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ADVANTAGES OF SOLID PARAFFIN FOR SEALING ANAEROBIC FLUID CULTURES

LEONARD R. THOMPSON

From the Clinical Laboratory, Napa State Hospital, and the Laboratory of Bacteriology and Experimental Pathology, Leland Stanford Junior University, California

In 1901, Park[1] recommended the use of solid paraffin for sealing anaerobic fluid cultures. Solid paraffin has been used subsequently by Weinzirl,[2] but apparently has not been adopted by other workers. It was the object of the present study to test the advantages of solid paraffin seals over the ordinary liquid paraffin method, and to devise a simplified solid paraffin technic.

TECHNIC

Preparation of Culture Tubes.—Paraffin melting at about 55 C. is heated well above the melting point, and delivered, in measured quantities, by means of a flame-heated pipet, into dry test tubes. With the ordinary half-inch test tube, 0.5 c c paraffin is sufficient. The tubes are plugged and sterilized in the upright position in an autoclave. After sterilization, the paraffin is allowed to solidify at the bottom of the tubes.

The liquid culture medium is now added to the tubes, and the tubes sterilized in an upright position by the discontinuous method. After each heating, the tubes are rapidly cooled by immersing them in cold water. After the final heating, the thoroughly cooled medium is stored until needed. The paraffin at this stage forms a solid layer from 2 to 5 mm. thick, at the top of each tube (fig. 1, A).

Inoculation of the Tubes.—The tube is gently warmed over a Bunsen flame, at the level of the paraffin plug. The plug can then be readily tipped with the platinum loop (fig. 1, B). After inoculation, the tube is again warmed slightly as before to seal the plug, and placed in an upright position in cold water for a few seconds.

Cleaning the Tubes.—To remove the paraffin after the tubes have been used, the cotton plugs are withdrawn, the tubes completely immersed in an upright position in water, and heated in a steam sterilizer. During the heating, the paraffin rises to the top of the water, from which it can readily be removed as a solid cake, after cooling.

CULTURAL ADVANTAGES

To test the advantages of the solid paraffin seal over the ordinary liquid paraffin method, parallel inoculations were made with stock anaerobic cultures, by the two methods. It was found that a tur-

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[1] Jour. Boston Soc. Med. Sci., 1901, 5, p. 373.

[2] Science, 1915, 42, p. 353.

bidity usually develops under the solid paraffin seal, sooner than under liquid paraffin. A comparison of this feature is shown in Figure 2, in which the inoculated tubes have been photographed against a wire screen to show relative turbidity.

It was further found that the percentages of positive cultures obtained with the tubes sealed with solid paraffin is greater than the percentage in the parallel inoculations by the liquid paraffin method.

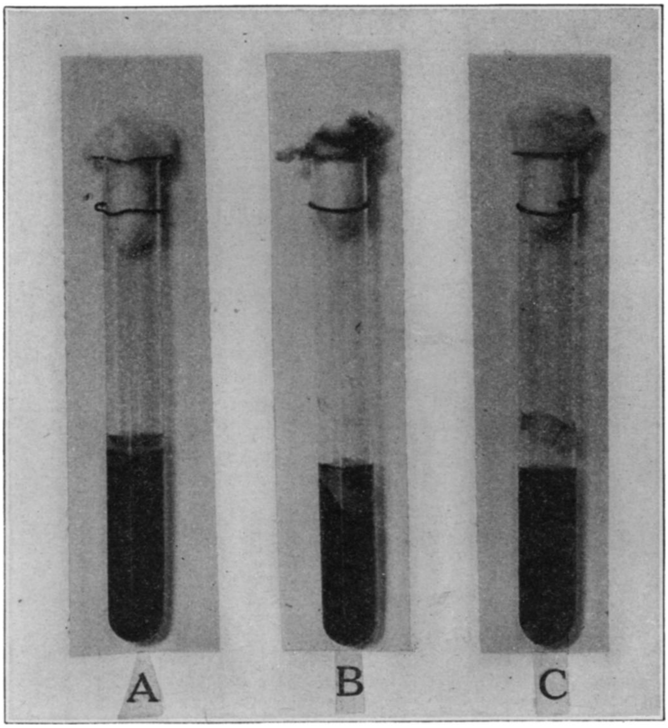

Fig. 1.—Solid paraffin seals. A, culture tube before and after inoculation. B, paraffin seal tipped during process of inoculation. C, paraffin seal lifted by gas formation.

This comparison is shown in table 1, in which 100% positive takes are recorded for the solid paraffin method, as contrasted with but 54% positive takes with the liquid paraffin method.

EXPLANATION OF ADVANTAGES

To determine the reasons for this advantage, tests were made of the relative rates of oxidation of colorless (heat reduced) litmus solution, by the two methods. Data from such tests are shown in figure 3.

From these data it is seen that slowly cooled, uncovered culture medium is oxidized within three hours, the oxygen entering the medium largely by downward convection currents during the process of cooling.

TABLE 1

COMPARISON OF SOLID PARAFFIN AND LIQUID PARAFFIN METHODS

Organism	Solid Paraffin			Liquid Paraffin		
	Inoculations	Growth	Percentages	Inoculations	Growth	Percentages
B. edematis.....	27	27	100	26	20	77
B. welchii.......	16	16	100	18	6	33
B. botulinus....	15	15	100	18	11	61
B. tetani........	16	16	100	18	6	33
Total........	74	74	**100**	80	43	**54**

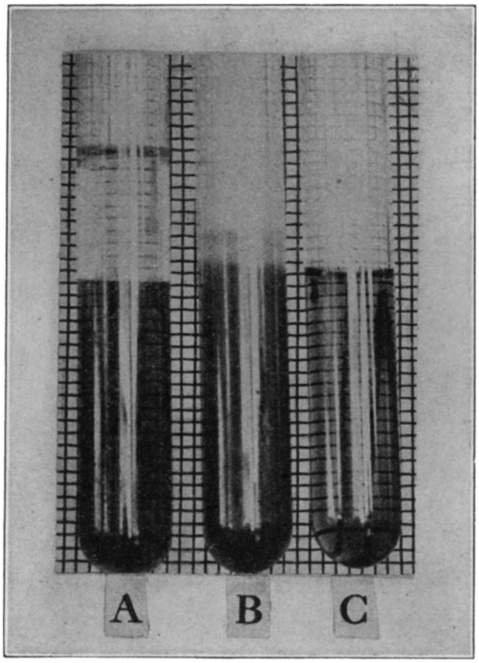

Fig. 2.—Comparative rapidity of growth under solid paraffin and liquid paraffin seals, ten hour stage, B. edematis. The tubes have been photographed against a wire mesh to show relative turbidity. A, culture under liquid paraffin seal, showing beginning turbidity in the lower portions of the tube. B, culture under solid paraffin seal, showing marked turbidity in the entire tube. C, aerobic control, showing no turbidity.

If uncovered medium is rapidly cooled, the entrance of oxygen is slower, the lower portions of the tubes remaining colorless for two or three days. Anaerobes will occasionally grow in rapidly cooled tubes, without a protecting layer of paraffin. A similar observation has been made by others.[3]

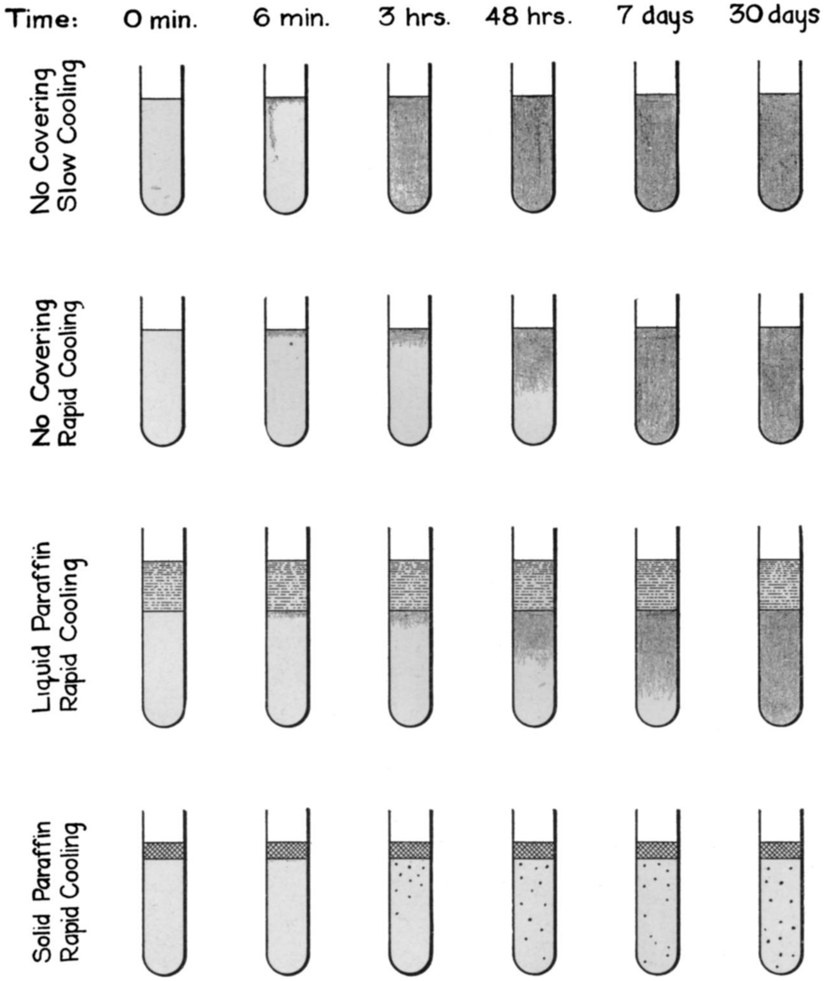
Time:
0 min.
6 min.
3 hrs.
48 hrs.
7 days
30 days
No Covering Slow Cooling
No Covering Rapid Cooling
Liquid Paraffin Rapid Cooling
Solid Paraffin Rapid Cooling

Fig. 3.—Relative rates of oxidation of heat reduced litmus. Shading shows distribution and relative intensity of the blue tint. In the solid paraffin tubes, a slight blue tint appears near the surface, during the few minutes before the paraffin becomes solidified. After solidification, there is no appreciable increase in the blue tint, even in tubes stored for 30 days. Handling, shaking and changes of temperature do not increase the rate of oxidation in these tubes.

If the rapidly cooled tubes are covered with liquid paraffin, they become oxidized much more slowly, the deeper portions of the tubes remaining colorless for from one to two weeks. If, however, these tubes are handled, shaken, or subjected to changes in temperature so as to set up convection currents, they may become blue within a few hours.

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[3] Rosenthal, C.: Compt. rend. Soc. Biol., 1906, 61, p. 440.

Rapidly cooled tubes, sealed with solid paraffin, take on a slight bluish tint near the surface, during the few minutes before the paraffin is solidified. After solidification there is no appreciable increase in the blue tint, even at the end of thirty days. Shaking, handling, or ordinary changes in temperature have no appreciable effects on the entrance of oxygen into these tubes.

SUMMARY

Tubes sealed with solid paraffin, by the method described, give a greater percentage of positive growths with stock anaerobic cultures than parallel tubes sealed with liquid paraffin.

This advantage is due to the prevention of the downward diffusion of oxygen by convection currents in the tubes sealed by the solid paraffin method.